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HOST INFLUENCE ON PLAQUE FORMATION BY VENEZUELAN EQUINE ENCEPHALITIS VIRUS

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ABSTRACT

In a study of the influence of the host on characteristics of Venezuelan equine encephalitis (VEE) virus, we have observed that virus populations propagated in the chick embryo, mouse brain, or monolayers of L cells differ in the following characteristics depending upon the host: (a) banding patterns in sucrose gradients, (b) ratio of soluble to insoluble lipids (in petroleum ether), and (c) size of plaques formed by both crude and purified virus when assayed in chick fibroblast monolayers. This report deals mainly with differences in plaque formation. The origin of the inoculum (chick embryo, mouse brain, or tissue culture) and the host (chick embryo, mouse brain, or tissue culture) both contributed to the determination of the ratio of large to small plaques obtained. VEE virus seeds prepared in each of the three hosts and used throughout this investigation were derived from the same equine strain. When chick embryo seed was used as inoculum, only large-plaque-forming virus was obtained from all three hosts. On the other hand, when mouse brain or tissue culture seeds were used as inocula, both large- and small-plaque-forming virus was obtained from all three hosts, with the plaque ratio being determined jointly by the inoculum and the host. Since Colón *et al*, in 1963, showed that the formation of small plaques by VEE virus is caused by the interaction of the virus with a sulfated polysaccharide inhibitor present in the overlay of the assay plates, we concluded that the large- and small-plaque-forming particle types differ in some surface characteristic or characteristics imposed upon them by the host.

A report by Heydrick and Wachter[1] showed that Venezuelan equine encephalitis (VEE) virus which had been purified by gradient centrifugation from chick embryo and from suckling mouse brain starting materials had essentially the same ratios of total lipid, phospholipid, and cholesterol; and that these ratios differed from those of the host materials. Further investigations of the influence of the host on the characteristics of VEE virus indicated that virus populations propagated in the chick embryo, mouse brain, or monolayers of L cells differ in the following characteristics depending on the host: (a) banding patterns formed in sucrose gradients, (b) ratio of soluble to insoluble lipids (in petroleum ether), and (c) the size of plaques formed by both crude and purified virus when assayed in chick fibroblast monolayers. This report will deal mainly with differences in plaque formation.

In 1962, Hearn and Soper[2] showed that an attenuated strain of VEE virus, which they had isolated, formed only minute plaques on chick fibroblasts, and that passage of this virus by the intracerebral route in mice or by the intraperitoneal route in hamsters produced a heterogeneous virulent virus population of mixed plaque types. These results indicated that the host exerted a strong influence on the characteristic of plaque formation by VEE virus.

In our experiments* we found that when a chick embryo seed was used as the inoculum to produce virus in chick embryo, suckling mouse brain, or monolayers of L cell, virus progeny formed only large plaques when assayed in chick fibroblast monolayers. When mouse brain seed was used as the inoculum for the same three hosts systems, virus progeny formed both large and small plaques in chick fibroblast monolayers. The egg and mouse seeds employed were both derived from the same master egg seed of VEE virus, the Trinidad strain of equine origin. The working egg seed we used had been through 14 passages in eggs, and the mouse brain seed through 13 passages in eggs and 5 passages in mice.

A number of experiments have been carried out to determine the relative influence of type of inoculum and type of host system on the ratio of large to small plaques formed by VEE virus in the chick fibroblast assay system. These results are shown in Table I. The results are in terms of a ratio of the number of large plaques to the number of small plaques formed in the assay plates after 48 hours of incubation. The large plaques were defined as those measuring three to five mm in diameter, and small plaques as those measuring one to two mm.

* In conducting the research reported herein, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

When the chick embryo inoculum, which contained virus that formed only large plaques was inoculated into hosts shown in Table I, the virus progeny also formed only large plaques. However, a virus inoculum with a plaque ratio of 1:3 from suckling mouse brain produced progeny in chick embryo which showed a plaque ratio of 1.4:1, a change in ratio toward large plaques. If this mouse brain virus was inoculated into L cell monolayers, the resulting progeny showed a plaque ratio of 1:11, a change in ratio toward small plaques. When this tissue culture virus with a plaque ratio of 1:11 was used as an inoculum, the virus from the embryo host again showed a plaque ratio change toward large plaques, the virus from the mouse brain host retained approximately the same ratio, but the virus from the L cell host system, (in this case the second passage) showed a further plaque ratio change toward small plaques. Table I shows that in an initial passage, embryo as a host influenced virus toward large plaque formation, suckling mouse brain did not initiate much of a change, and an L cell tissue culture influenced virus toward small plaque formation.

TABLE I. EFFECT OF LABORATORY HOST ON PLAQUE FORMATION BY VEE VIRUS IN CHICK FIBROBLAST MONOLAYERS

Inoculum Virus	Host Material		
	Embryo	Mouse Brain	L Cell Tissue Culture
Chick Embryo (L)	L[a]	L	L
Mouse Brain (1:3)	1.4:1[b]	1:3	1:11
L Cell Tissue Culture (1:11)	1:2.5	1:13±1	1:20±3

a. All large plaques.
b. Ratio of large to small plaques.

Table II shows the results when the same virus seeds were inoculated into three tissue culture hosts. Virus produced in these three hosts from the embryo inoculum again showed all large plaques. Virus produced from the mouse brain inoculum showed a marked tendency toward small plaque formation. Virus produced from the L cell tissue culture inoculum in the chick fibroblast host formed only small plaques.

TABLE II. EFFECT OF DIFFERENT TISSUE CULTURE SYSTEMS ON PLAQUE FORMATION BY VEE VIRUS IN CHICK FIBROBLAST MONOLAYERS

Inoculum Virus	Host Cell Monolayer		
	Guinea Pig Lung	Chang's Liver	Chick Fibroblast
Chick Embryo (L)	L[a]	L	L
Mouse Brain (1:3)	1:18±2[b]	1:15±1	1:23±2
L Cell Tissue Culture (1:11)	-	-	S[c]

a. All large plaques.
b. Ratio of large to small plaques.
c. All small plaques.

In 1962, Marshall, Scrivani, and Reeves[3] reported variation in the size of plaques produced in tissue culture by strains of western equine encephalitis (WEE) virus. Some of their findings with WEE virus parallel those we have observed for VEE virus. The large-plaque characteristic Marshall et al observed in naturally occurring strains of WEE virus was reliably consistent during passage through embryonated eggs, less consistent in mouse-brain passage, and was quickly replaced by virus that produced small plaques when passed through chick fibroblast tissue culture. In addition, Marshall and co-workers showed that with two wild strains of WEE virus, nearly complete change from large to small or intermediate plaque character occurred at the second passage level in chick fibroblast tissue culture.

We have also found that the influence of the host used for passage applies also to the maintenance of the large-plaque character of VEE virus. VEE virus from chick embryo that produced all large-plaque-forming virus on initial passage in all the hosts studied was serially passed to observe the host influence on the large-plaque characteristic. The results of such passages in five hosts are shown in Table III. The results are indicated as the percentage of large plaques as seen in each passage. Note that the embryo host maintained the large plaque virus through five passages; however, the suckling mouse brain host showed a gradual decrease in large plaques until the fifth passage when a sharp drop in large plaque number was seen. Among tissue culture hosts the guinea pig lung system showed a gradual reduction of large-plaque-forming virus through the fifth passage, whereas the L cell and chick fibroblast systems caused a rapid reduction in the percentage of large-plaque-forming virus, so that by passages four and five only about two per cent large-plaque-forming virus remained.

TABLE III. HOST INFLUENCE ON PLAQUE FORMATION BY VEE VIRUS OF CHICK EMBRYO ORIGIN

Host Material	Passage Number 1	2	3	4	5
Chick Embryo	100[a]	100	100	100	100
Suckling Mouse Brain	100	100	90	85	35
L Cell	90	55	25	3	1
Guinea Pig Lung	95	90	85	72	50
Chick Fibroblast	100	80	12	2	2

a. Per cent large plaques formed in chick fibroblast monolayers.

When DEAE-dextran was incorporated, at a concentration of 0.1 mg per ml, into the agar overlay of the chick fibroblast assay plates, a conversion of small plaques to approximately the size of large plaques was obtained. Typical examples of plaque counts obtained with and without DEAE-dextran added to assay plates are shown in Table IV. The values listed represent the actual count of large and small plaques on individual assay plates of the same dilution of sample. As indicated previously, the chick embryo inoculum contained only large-plaque-forming virus, and addition of DEAE-dextran to assay plates of this type of virus caused no change in plaque size. When mouse brain and tissue culture inocula containing both large- and small-plaque-forming virus were assayed, small plaques were seen in assay plates that had no DEAE-dextran in the agar overlay, but only large plaques in parallel plates that contained DEAE-dextran. As is indicated by these results with DEAE-dextran, the presence of an inhibitor in the overlay is directly responsible for the small plaque characteristic. This observation is based more specifically on the report of Colón, Idoine, and Brand[4] who have shown that differences in plaque size among preparations of the equine encephalitis viruses is due to the presence of an inhibitor in agar, and that this inhibitor combines with the small-plaque-forming virus and not with the large-plaque-formers. Based on this information, and assuming that the interaction of inhibitor and virus occurs at the surface of the virus, we concluded that the large-plaque- and small-plaque-forming virus differ in some surface characteristic or characteristics imposed upon the virus particle by the host.

TABLE IV. EFFECT OF DEAE-DEXTRAN ON PLAQUE FORMATION BY VEE VIRUS IN CHICK FIBROBLAST MONOLAYERS

Inoculum Virus	Examples of Large to Small Plaque Count Without DEAE-Dextran	With DEAE-Dextran
Chick Embryo	163:0	123:0
Mouse Brain	15:62	134:0
L Cell Tissue Culture	14:200	265:0

Attempts were made to separate the large-plaque-forming virus from the small-plaque-forming virus on the basis of sucrose density gradient centrifugation using the procedure described in an earlier paper.[1] Although differences in banding patterns were seen for different hosts, material that included large- and small-plaque-forming virus produced bands that contained virus of both plaque types. Shown from left to right in Figure 1 are gradients of VEE virus purified from chick embryo, suckling mouse brain and tissue culture hosts. Note that embryo virus gave a sharply defined lower virus band, with a visible, but less distinct upper virus band, while the suckling mouse brain virus gave two sharply defined bands and the tissue culture virus gave one sharply defined band.

Another characteristic noted was that purified VEE virus derived from chick embyro possesses a ratio of petroleum ether-soluble to -insoluble lipid that is different from that of purified VEE virus derived from mouse brain. The extent of this lipid difference and a summary of other differences that were discussed are presented in Table V. As indicated before, large-plaque-forming virus does not interact with agar inhibitor. However, the small-plaque-forming virus does interact with agar inhibitor, and the formation of small plaques is dependent on this interaction. Therefore the characteristic listed as "Interaction with Inhibitor" is not a distinct and separate characteristic but is directly related to the item "Plaque Ratio." To repeat, no relationship was found between plaque ratios and banding patterns in sucrose gradients. In reference to the difference in lipid solubility, note that virus from chick embryo has a ratio of petroleum ether-soluble to -insoluble lipid of 1.5:1 but that mouse brain-derived virus has the inverse of this, a ratio of 1:2. For purified virus from tissue culture initial results indicate that the ratio is approximately 1:4. Information on lipid solubility is presented to suggest that there may be some correlation between differences in solubility of lipids of VEE virus from various hosts and differences in plaque ratio. In this regard, it is of interest that selective and reversible binding of serum lipoproteins by sulfated polysaccharides has been reported by Burstein.[6]

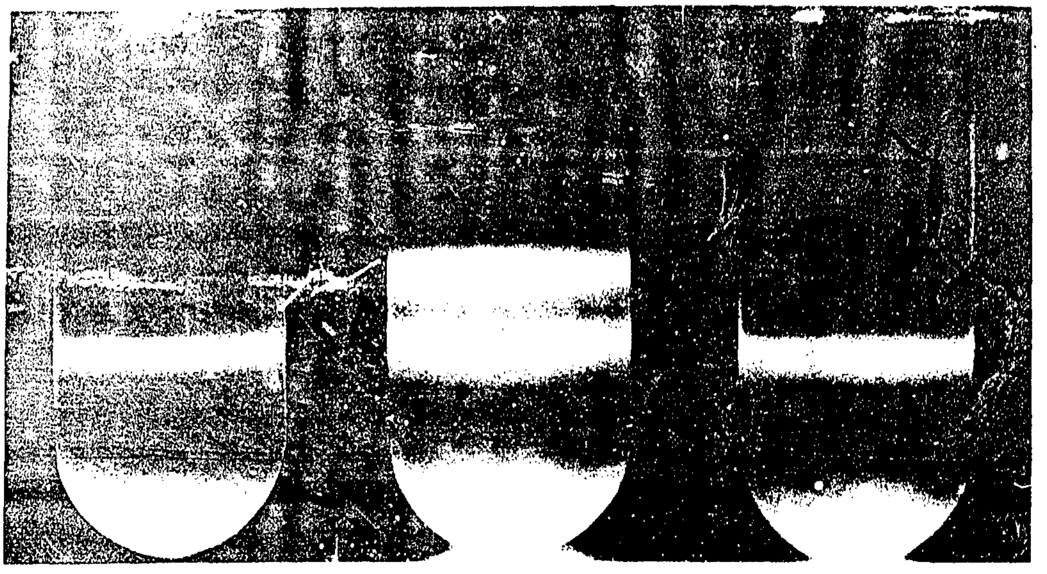

Figure 1. Comparison of Gradient Bands of Purified VEE Virus. (From left to right: chick embryo, suckling mouse brain, and tissue culture.)

In summary, it has been found that with VEE virus, the characteristics of plaque size, lipid solubility, and banding patterns in gradients depend upon the derivation of the virus. Our studies suggest that the results of characterization of viruses in general should be interpreted in the light of the origin of the inoculum and the nature of the host itself.

TABLE V. OBSERVED DIFFERENCES FOR VEE VIRUS PROPAGATED IN DIFFERENT HOST SYSTEMS

Host System	Interaction with Inhibitor[a]	Plaque Ratio[b]	Bands in Gradient	Lipid Ratio[c]
Chick Embryo	No	L[d]	2	1.5:1
Mouse Brain	Yes	1:3	2	1:2
Tissue Culture	Yes	1:11	1	1:4

a. Interaction of virus particle with the inhibitor from agar.
b. Ratio of large to small plaques.
c. Ratio of petroleum ether-soluble to petroleum ether-insoluble lipid.
d. All large plaques.

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